

BIOENGINEERED TISSUE CONSTRUCTS AND METHODS FOR PRODUCING AND USING THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 61/347,725, filed on May 24, 2010, U.S. Provisional Application No. 61/337,938, filed on February 12, 2010, and U.S. Provisional Application No. 61/295,073, filed on January 14, 2010; the entire contents of each of which are expressly incorporated herein by reference.

BACKGROUND OF THE INVENTION

Bone, cartilage, tendon, ligament, muscle, adipose, and marrow stroma are examples of mesenchymal tissues (*i.e.*, tissues that differentiate from mesenchymal stem cells).

Mesenchymal tissues may be injured during surgery or they may develop disease from a genetic disorder or environmental perturbation.

Accordingly, new therapies for repairing diseased or damaged tissues are needed.

SUMMARY OF THE INVENTION

In one aspect, the present invention refers to a method for producing a bioengineered construct having an extracellular matrix with an engineered average pore size comprising:

a) seeding cells capable of synthesizing extracellular matrix components within a culture vessel;

b) culturing the cells to synthesize, secrete and organize extracellular matrix components;

c) determining the desired average pore size to be engineered;

d) lyophilizing at least the resulting extracellular matrix components, wherein lyophilization comprises freezing the extracellular matrix components to a final freezing temperature of between about -100°C to about 0°C, wherein the average pore size of the porous extracellular matrix increases as the final freezing temperature increases and subsequently drying the extracellular matrix components, thereby producing a bioengineered extracellular matrix construct having an extracellular matrix with the engineered average pore size.

Featured herein are bioengineered constructs comprising extracellular matrix (ECM) in forms., which are optimized for particular therapeutic uses.

In another aspect, the present invention refers to a bioengineered construct comprising:
extracellular matrix-producing cells;
endogenous extracellular matrix produced by the extracellular matrix-producing cells;
wherein the extracellular matrix-producing cells are (a) devitalized (b) the

5 bioengineered construct has pores in the range between 10 microns and 150 microns in
diameter, and (c) the bioengineered construct is formed by cells cultured in chemically
defined media, optionally wherein the pores are in the range between 50 microns and 100
microns or between 80 microns and 100 microns, and/or the bioengineered construct is in
powdered form.

10 In another aspect, the present invention refers to a bioengineered construct
comprising mesenchymal stem cells grown under chemically defined media conditions that
are free of undefined animal organ or tissue extracts to produce a layer of extracellular matrix,
which is synthesized and assembled by the mesenchymal stem cells.

Certain constructs are comprised of extracellular matrix produced by cultured
15 mesenchymal stem cells (MSCs). Certain constructs also comprise the cells that produce
the matrix. In certain constructs, the cells have been devitalized. In other constructs, the
cells, which produce the extracellular matrix have been removed to produce
decellularized constructs.

Certain constructs have a thickness of at least about 30 μm . Certain constructs
20 include pores having an average diameter in the range of 10 to 100 μm . Certain constructs
have an average F_{max} of at least 0.4 Newtons. Certain constructs have an ultimate tensile
strength (UTS) of at least 0.4 Megapascals. Certain constructs have a plastic deformation
tolerance of at least 0.4 times the initial length.

The ECM in the constructs may be further processed (*e.g.*, dehydrated, crosslinked,
25 contracted, micronized, sterilized, etc.) or further combined with other biologically active
substances or support materials (*e.g.*, silk, an adhesive, etc.) for preparation of therapeutic
products.

In another aspect, the present invention refers to a multilayered bioengineered
construct, wherein at least two bioengineered constructs as defined above are bonded
30 together, optionally wherein the bonded bioengineered constructs are crosslinked with a
crosslinking agent.

Further featured are methods for making and modifying the bioengineered